

REMARKS

Claims 49 and 58–100 are pending in this Application. The Applicant has cancelled claims 50-57 without prejudice to his rights to pursue the subject matter of these claims in this or other applications. The Applicant has added new claims 58 - 100 which more clearly define the subject matter of the invention and properly fall within the subject matter of the elected claims. Support for the newly added claims is found throughout the specification and/or in the claims as originally filed.

The limitation of cancelled claim 50, wherein blood samples of test and control subjects have not been fractionated into cell types, has been incorporated into new independent claims 60, 61, 62, 69, 70, 71, 78, 79, and 80. New claims 63, 64, and 65 have been added, essentially corresponding to amended claim 49, and new claims 58 and 59, respectively, but with the recitation “*blood samples which comprise leukocytes which have not been fractionated into cell types*”. Newly added claims 63, 64 and 65 have been added in order to clarify that the RNA is of blood samples which include all of the types of leukocytes in whole blood, i.e. of blood samples which include granulocytes in addition to mononuclear cells (T-lymphocytes, B-lymphocytes and monocytes). This phrase finds clear support in the specification, including at Figure 5C which shows standardized levels of insulin gene in each of the fractions of leukocytes which collectively constitute unfractionated leukocytes, i.e. granulocytes, T-lymphocytes, B-lymphocytes and monocytes (labeled “G.R.”, “CD 3+”, “CD19” and “MONO”, i.e., respectively). It is well known to the ordinarily skilled artisan that CD3 and CD19 are specific cell surface markers of T-lymphocytes and B-lymphocytes (refer, for example, to the enclosed Abstract of Casey *et al.*, 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9). The fact that granulocytes (G.R.), lymphocytes [T-lymphocytes (CD 3+) and B-lymphocytes (CD19+)] and monocytes (MONO) represent all of the types of leukocytes found in blood is evident in Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. Fig. A.23, of record., which clearly teaches that leukocytes are

composed of granulocytes and mononuclear cells, and that the latter are composed of lymphocytes and monocytes. Additional support for the term “leukocytes” is found at paragraph [0005] of the published application US 2007/0031841 (hereinafter the “Published Application”).

New claims 66-74 have been added which are directed to a method of merely testing for coronary artery disease in a human test subject. The claims correspond to amended claim 49, and new claims 58-65, respectively. The claims as amended are supported by the specification and in particular are supported at paragraph [0013]. New claims 75-83 have been added which are directed to a method of detecting expression of a CRTAM gene. The claims correspond to newly amended claim 49, and new claims 58-65, respectively. The claims as amended are supported by the specification and in particular are supported at paragraph [0013], paragraph [0057], Table 3L and Figure 19.

The limitation of cancelled claim 52, wherein quantification of test subject RNA levels is effected relative to a housekeeping gene, has been incorporated into new claim 101. The limitation of cancelled claim 53, wherein quantification of test subject RNA levels is effected by quantifying cDNA has been incorporated into new claim 94 and 97. The limitation of cancelled claim 54, wherein comparison of RNA levels of a test subject and of control subjects not having coronary artery results in determination of a statistically significant difference has been incorporated into new independent claims 58, 59, 61, 62, 64, 65, 67, 68, 70, 71, 73, 74, 76, 77, 79, 80, 82 and 83. The limitation of cancelled claim 55, wherein comparison of RNA levels of a test subject and of control subjects having coronary artery results in determination of a statistically significant similarity has been incorporated into new independent claims 58-83. The limitation of cancelled claim 56, wherein quantifying of test subject RNA levels is effected using quantitative PCR, has been incorporated into new claim 95 and 99. Support for the limitation wherein biomarker RNA levels of a test subject and of **healthy control subjects** are compared can be found in the published application (US 2004/0265868), for example, at Example 21, including at paragraphs [0364] (“... *compared to blood samples taken from healthy patients*”) and [0367] (“*Identification of genes differentially expressed in blood samples from patients with Coronary artery disease as compared to healthy*”).

patients...”); and at paragraph [0129] (“... *patients without disease or healthy patients...*”). Support for the limitation wherein comparison of biomarker RNA levels of a test subject and of control subjects having coronary artery disease and/or of control subjects not having coronary artery disease results in determination that there is a statistically significant similarity and/or statistically significant difference, respectively, so as to be indicative of coronary artery disease in the test subject can be found in the published application, for example at paragraphs [0107] (“*when comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true*”), [0108] (“*when comparing two or more samples for differences, results are reported as statistically significant when there is only a small probability that similar results would have been observed if the tested hypothesis (i.e., the genes are not expressed at different levels) were true*”), [0134] (relating to “*methods that can be used for class prediction analysis*”), [0366] (“*Blood samples were taken from patients who were diagnosed with Coronary artery disease as defined herein. Gene expression profiles were then analysed and compared to profiles from patients unaffected by any disease.*”) and [0368], describing the CAD vs healthy control clustering data shown in Figure 19. No new matter has been entered.

35 U.S.C. § 112, 2nd Paragraph, Rejections – Indefiniteness

Claims 51-57 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to point out and distinctly claim the subject matter which the Applicant regards as the invention. More particularly, the phrase “unfractionated samples of lysed blood” has been objected to.

Applicant respectfully traverses the rejection, including as it would apply to any of the newly added and/or amended claims. Applicant submits that the many embodiments of blood samples disclosed in the specification do not render the referenced phrase indefinite. However, solely for the purposes of expediting prosecution, Applicant has now cancelled claims 51, 52, 53, 54, 55, 56 and 57, without prejudice to his rights to pursue the subject matter of these claims in this or any other application

In order to more clearly set forth the claimed subject matter, Applicant has now added new independent claims 63, 64 and 65 which recite the phrase “*blood samples which comprise leukocytes which have not been fractionated into cell types*”.

Applicant respectfully submits that the phrase “blood samples which **comprise leukocytes which** have not been fractionated into cell types” finds clear support in the specification. As noted above, support is found throughout the specification including at Figure 5C, which shows the fractionation of different types of leukocytes which collectively constitute unfractionated leukocytes, i.e. granulocytes, T-lymphocytes, B-lymphocytes and monocytes (labeled “G.R.”, “CD 3+”, “CD19” and “MONO”, i.e., respectively). It is well known to the ordinarily skilled artisan that CD3 and CD19 are specific cell surface markers of T-lymphocytes and B-lymphocytes (refer, for example, to the enclosed Abstract of Casey *et al.*, 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9). The fact that granulocytes, lymphocytes (T-lymphocytes and B-lymphocytes) and monocytes represent all of the types of leukocytes found in blood is evident in Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. Fig. A.23, (enclosed), which clearly teaches that leukocytes are composed of granulocytes and mononuclear cells, and that the latter are composed of lymphocytes and monocytes. The ordinarily skilled artisan will readily understand, particularly in view of the legend of Figure 5C which recites “*FIG. 5C shows... each fractionated cell from whole blood.*”, that data representing each type of blood leukocyte is represented in Figure 5C; that “G.R.” is an acronym representing granulocytes; that “CD 3+” refers to CD3+ cells, i.e. T-lymphocytes; that “CD19” refers to CD19+ cells, i.e. B-lymphocytes; that “MONO” is an acronym representing monocytes; and that all lymphocytes are represented in Figure 5C since whole lymphocytes consist of T- and B-lymphocytes. Literal support for the term “leukocytes” can be found at paragraph [0005] of the published application.

In view of this amendment and remarks clarifying the claimed embodiments, Applicant respectfully requests that these rejections be reconsidered and withdrawn.

35 U.S.C. § 112, 1st Paragraph Rejections, - Written Description

Claim 55-57 are rejected under 35 U.S.C. § 112, first paragraph, as failing to comply with the written description requirement.

The office action states that the limitation "unfractionated samples of lysed blood" appears to be new matter. Applicant traverses the rejection, but has now removed the referenced phrase from the claims, solely for the purposes of advancing prosecution.

As described above in the response to the 35 U.S.C. § 112, 2nd paragraph rejections, Applicant has now added new independent claims 63, 64 and 65 which, for greater clarity, recite the phrase "blood samples which comprise leukocytes which have not been fractionated into cell types", which finds clear written support in the specification, as explained above.

In view of this amendment and remarks, Applicant respectfully requests that this rejection be reconsidered and withdrawn.

35 U.S.C. § 112, 1st Paragraph Rejections, - Enablement

Claims 49-57 are rejected under 35 U.S.C. 112, first paragraph, as failing to comply with the enablement requirement. The Applicant respectfully traverses the rejections.

Nature of the Invention and Scope of claims

The office action dated April 16, 2007 (hereinafter "Office Action") states:

"Thus, the independent claim, as written, states that a comparison of a human test subject CRTAM RNA level in a blood sample to a control indicates that coronary artery disease is present in the test subject." (p. 4)

"The claims are extremely broad because they require set forth that any or all comparison between a test subject and RNA level from "control subjects" is indicative of disease." (p. 5), and

“The claims are broad with regard to control subjects would could encompass patients with coronary artery disease, healthy patients, patients with some other disease, such as large granular lymphocyte leukemia or bladder cancer, patients with a particular stage of coronary artery disease, etc...” (p. 5)

The Applicant respectfully disagrees that the claims set forth that any or all comparison is sufficient to indicate the presence of coronary artery disease in the test subject, and that the claimed control subjects may encompass patients having coronary artery disease, healthy patients, and patients with some other diseases such as large granular lymphocyte leukemia, bladder cancer, or a particular stage of coronary artery. Nevertheless, solely to expedite prosecution, Applicant has now amended independent claim 49 and has added new independent claims 58-65 so that **each of the independent claims requires** a step of comparing levels of CRTAM RNA in blood samples between the test subject and control subjects having coronary artery disease, and determining a **“statistically significant similarity” between the levels.**

Thus, for example, newly added independent claims 58, 61 and 64 require, to indicate coronary artery disease in the test subject, a comparison which must result in **both (i) a “statistically significant similarity” between the level of RNA in the blood sample of the test subject as compared with the level of RNA in blood of the control subjects having coronary artery disease and (ii) a “statistically significant difference”** between the level of RNA in the blood sample of the test subject as compared with the level of RNA in blood of the control subjects not having coronary artery disease in order to be indicative of coronary artery disease in the test subject.

Similarly, newly added independent claims 59, 62 and 65 require, to indicate coronary artery disease in the test subject, a comparison result in a determining that there be a “statistically significant difference” between the level of RNA in the blood sample of the test subject as compared with the level of RNA in blood of healthy control subjects.

Thus, the instant claims clearly do not set forth that the comparison alone, no matter the result of the comparison, is sufficient to indicate coronary artery disease as suggested. Instead, to indicate coronary artery disease in the test subject, the comparison of the levels of the test subject with those of positive control subjects having coronary

artery disease must result in a determination of a similarity. Some of the claims also require an additional comparison with negative control subjects (either healthy individuals or individuals not having coronary artery disease). These comparisons must result in determination of a difference between the levels. Furthermore, the similarity or difference must be one with a statistical degree of significance, which may be determined, for example, by the many statistical techniques widely used in assessing the use of specific biomarkers in diagnosis, including those statistical techniques referenced in the instant specification, and incorporated by reference. Applicant further respectfully submits that, as explained below, the instant claims clearly address the Examiner's concerns at p. 5 of the Office Action that the claims may encompass that any level and direction of difference in gene expression in blood samples between a test subject and control subjects is indicative of disease.

Furthermore independent claims 59, 62 and 65 require comparing levels of CRTAM RNA in a blood sample of a test subject to levels of CRTAM RNA **in healthy control subjects**, and independent claims 58, 61 and 64 require comparing levels of CRTAM RNA in blood sample of a test subject to **levels of CRTAM RNA in control subjects not having coronary artery disease**. Thus, the instant claims clearly do not set forth "control subjects" which may encompass patients with coronary artery disease, healthy patients, patients with some other disease, such as large granular lymphocytic leukemia or bladder cancer, patients with a particular stage of coronary artery disease etc., as suggested at p.5 of the Office Action. Rather, each type of control subjects recited are subjects which either have coronary artery disease, do not have coronary artery disease, or are healthy.

The Applicant has also added new claims 66 – 74 which are directed to a method of merely testing a human test subject for coronary artery disease. **These claims do not additionally require that the test results in an indication that coronary artery disease is present in the test individual.** Rather, in accordance with claims 66, 69, and 72, the test requires a comparison of the quantified level of RNA encoded by CRTAM in the test subject with the level of RNA encoded by CRTAM in the control subjects having coronary artery disease results in a determination of a statistically significant similarity.

Similarly, in accordance with claims 67, 70, and 73, the test requires a comparison of the quantified level of RNA encoded by CRTAM in the test subject with the level of RNA encoded by CRTAM in control subjects not having coronary artery disease and with the level of RNA encoded by CRTAM in control subjects having coronary artery disease, and these comparisons must result in a statistically significant difference and a statistically significant similarity, respectively. Finally, in accordance with claims 68, 71 and 74, the test requires a comparison of the quantified level of RNA encoded by CRTAM in the test subject with the level of RNA encoded by CRTAM in healthy control subjects and with the level of RNA encoded by CRTAM in control subjects not having coronary artery disease, and these comparisons must result in a statistically significant difference and a statistically significant similarity, respectively.

Finally the Applicant has added new claims 75-83 which are directed to a method of detecting the expression of a CRTAM gene in a human test subject. **These claims also do not additionally require that the method results in an indication that coronary artery disease is present in the test individual.**

Level and Magnitude of Differential Expression

The Office Action states that the claims do not set forth the directionality and magnitude of the difference in CRTAM RNA levels in blood samples between a test subject and control subjects, which would be necessary to indicate coronary artery disease in the test subject.

Applicant respectfully submits, however, that the claims need not set forth the direction and magnitude of the differential gene expression for enablement of the claims. The fact that each of the claims requires determination of a statistically significant similarity between the test subject and control subjects having coronary artery disease makes it unnecessary to include the direction and magnitude because no direction or magnitude information is required in order to compare for similarity.

Furthermore, even to satisfy the further requirement in claims 58, 59, 61, 62, 64, 65, 67, 68, 70, 71, 73, 74, 76, 77, 79, 80, 82, and 83 of a statistically significant

difference between the test subject and the stated control subjects, the Applicant submits that CRTAM RNA levels in blood of subjects having coronary artery disease and of subjects not having coronary artery disease are inherent biological features of such subjects which are readily determined without undue experimentation. This is clearly demonstrated in the enclosed Declaration under 37 CFR 1.132 (hereinafter "the Declaration") which discloses post-filing experiments performed which continue to demonstrate that there is statistically significant differential expression as between patients having coronary artery disease and healthy control patients. The results of these experiments demonstrate that the average level of CRTAM-encoded RNA in blood samples from 19 coronary artery disease patients, as determined via quantitative RT-PCR, is about 2.9-fold higher than that of 14 healthy control subjects not, with the difference in expression levels being statistically significant ($p < 0.05$). Thus, the magnitude and directionality of the difference in levels of CRTAM RNA in blood of subjects having coronary artery disease relative to subjects not having coronary artery disease is an inherent biological feature of such subjects which does not need to be set forth in the claims for enablement.

The experimental results disclosed in the Declaration merely validate the teachings of the specification. The Applicant respectfully submits that the invention is taught by the specification and claimed in such terms that one skilled in the art can make and use the claimed invention, including the use of the elected biomarker CRTAM as an indicator of coronary artery disease without disclosing the direction or the level of difference that exists between patients having coronary artery disease and individuals not having coronary artery disease. The Applicant has identified the elected gene CRTAM as differentially expressed as between individuals diagnosed as having coronary artery disease and individuals not having coronary artery disease by demonstrating a statistical difference in the level of RNA, as described in Example 21. The statistical significance of CRTAM's differential expression is evidenced by its P- value of 0.029 as listed in Table 3L, acknowledged by the Office Action. The dendrogram of Figure 19 demonstrates that the CRTAM gene is one of a number of genes which demonstrate a statistically significant difference as between a population of 2 individuals who have coronary artery disease and 21 individuals not having coronary artery disease. Therefore

the Applicant has taught that there is a significant difference in differential expression for CRTAM as between a population of individuals having coronary artery disease and a population of individuals not having coronary artery disease, and further has taught to compare the level of expression of CRTAM in a test individual with populations having coronary artery disease and populations not having coronary artery disease, such as healthy subjects, using classification methods to determine the similarity or difference in gene expression levels as between the test subject and the tested control populations. Support for such teachings can be found in the published application, for example at paragraphs [0126] to [0127] (relating to “...comparing two or more samples for differences...”), paragraph [0128] (relating to “*Identification of genes differentially expressed in blood samples from patients with disease as compared to healthy patients or as compared to patients without said disease...*”), [0133] (relating to utilization of “...sets of genes which have been identified as statistically significant” in order to “...characterize an unknown sample as having said disease or not having said disease...”) for class prediction, [0134] (relating to “*Methods that can be used for class prediction analysis...*”), [0135] (relating to use of algorithms which “...can be used to query additional samples against the existing database to further refine the diagnostic and/or prognostic determination by allowing an even greater association between the disease and gene expression signature...”), and [0367] (relating to “*Identification of genes differentially expressed in blood samples from patients with Coronary artery disease as compared to healthy patients... by statistical analysis using the Wilcox Mann Whitney rank sum test ...*”).

All of the instant claims require that the level of expression of RNA corresponding to CRTAM be compared with the level of CRTAM in other individuals who have coronary artery disease and require at minimum a statistically significant similarity as between the test subject and control subjects having coronary artery disease before the level of gene expression of CRTAM is considered to be indicative of coronary artery disease. Furthermore, the Applicant contends that it does not require undue experimentation for one of skill to determine the inherent direction or level of the statistically significant differential expression required for the claimed methods of detecting coronary artery disease, given the widely established and validated analytical

tools for analyzing gene expression levels. As such, it is not necessary for the claims to set forth the direction or magnitude of difference between CRTAM levels in blood samples from a test subject and control subjects not having coronary artery disease (such as healthy subjects). The Applicant has provided sufficient information by teaching that there is a statistically significant difference and that it is significant as between the populations. Given the widely established and validated analytical tools for analyzing gene expression levels, and the reduction to practice of the similar experiment within the Published Application, this type of experimentation qualifies as routine experimentation and therefore is not undue ("The test is not merely quantitative, since a considerable amount of experimentation is permissible, if it is merely routine, or if the specification in question provides a reasonable amount of guidance with respect to the direction in which the experimentation should proceed." In re Wands, 858 F.2d 731, 737, 8 USPQ2d 1400, 1404 (Fed. Cir. 1988) (citing In re Angstadt, 537 F.2d 489, 502-04, 190 USPQ 214, 217-19 (CCPA 1976)).

Predictability

The Office Action states that Lee teaches that data obtained from gene chips must be replicated in order to screen out false positive results; Cheung et al. (2003) teaches that there is natural variation in gene expression amongst different individuals; Wu et al (2001) teaches that gene expression data, such as microarray data, must be interpreted in the context of other biological knowledge, and that the conclusions that can be drawn from a given set of data depend on the particular choice of data analysis; and Newton et al. (2001) teaches that a replication of data is required for validation (p.9 Office Action).

The Applicant specifically addresses the Examiner's concerns regarding Lee; Cheung et al.; Wu et al and Newton et al, as described above, with the provision of the Declaration which clearly indicates, using additional samples and additional techniques (e.g. quantitative RT-PCR), that CRTAM continues to demonstrate differential expression as between populations of individuals having coronary artery disease and populations of individuals not having coronary artery disease.

Furthermore, the Applicant would point out that the results disclosed by Cheung *et al.* cannot be reliably extrapolated to primary blood samples since the lymphoblastoid cells employed by Cheung *et al.* are significantly modified relative to primary blood cells, due to being cultured cell lines generated by immortalization of primary human cells derived from “CEPH” families, as indicated in Reference no. 10 of Cheung *et al.* (Dausset *et al.*, 1990. Genomics 6:575; enclosed) at p. 575, right column, 1st paragraph. Applicant wishes to point out that immortalized cultured cell lines such as the lymphoblastoid cells taught by Cheung *et al.* undergo significant genetic modification such as strong genome-wide demethylation (refer, for example, to enclosed abstract of: Vilain *et al.*, 2003. DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogenet Cell Genet. 90:93), as a result of extensive *in-vitro* culturing in the absence of immune or apoptotic mechanisms which function to eliminate mutated cells in the body. As such, immortalized CEPH lymphoblastoid cells may represent a particularly unsuitable cell type for modeling gene expression variability in primary blood cells.

Applicant would also respectfully disagree with the contention in Wu *et al.* that expression data needs to be interpreted in view of other biological knowledge. Differential gene expression which is reproducible, and is correlated with the state of health or disease of the individual does not necessarily result directly from the state of disease of the individual. Rather these changes in expression can be as a result of a downstream effect of pathogenic processes, and it is not necessary that the biological relevance of the data be known to allow this difference in expression to be useful as a biomarker. For example prostate-specific phosphatase and prostate-specific antigen (PSA) were long used as biomarkers without an understanding of their function (refer, for example, to the enclosed abstracts of: Chu TM, 1990, (Prostate cancer-associated markers. Immunol. Ser. 53:339-56; and Diamandis EP., 2000. Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 46:896-900).

As stated in the Manual of Patent Examining Procedure at 2164.03: the “predictability or lack thereof” in the art refers to the ability of one skilled in the art to extrapolate the disclosed or known results to the claimed invention. In this case the disclosed result is a statistically significant difference in the level of CRTAM RNA as

between individuals having coronary artery disease and individuals not having coronary artery disease. The claimed invention requires a statistically significant similarity between the level of expression of CRTAM between the test subject and individuals having coronary artery disease and a statistically significant difference as between the test subject and individuals not having coronary artery disease. These results are confirmed by the Declaration. One skilled in the art can readily anticipate utilizing similar experiments to those described in the specification, and applied in the Declaration, to the claimed invention.

Specificity of the Elected Biomarker

The Office Action states that the “*there is no analysis of all possible diseases or phenotypes to determine if the gene expression difference observed in the instant application is specific to coronary artery disease such that any difference between a test patient and blood samples from control subjects is sufficient to conclude coronary artery disease is present*” (p.7 of the Office Action). The Office Action cites Loughran, JR et al. as observing that “*CRTAM is differentially expressed in patients having large granular lymphocyte leukemia as compared to healthy patients*” (US 2007/0020666, p.23), and Zlongtnik et al. as teaching that CRTAM is expressed specifically on activated class I MHC-restricted T cells (p.7-8 of the Office Action).

The Applicant respectfully disagrees with the Examiner that the instantly claimed invention requires a conclusion that coronary artery disease is present to the exclusion of all other possible diseases or phenotypes. The Applicant would note that such an extremely high burden is unreasonable for the invention as claimed in any of claims 49 and 58-65 which merely require a determination of statistically significant similarity and/or statistically significant difference which is indicative of coronary artery disease. Furthermore, new claims 66-75 merely disclose a test which results in a statistically significant similarity and/or statistically significant difference and new claims 76- 83 are directed to methods of detecting expression of CRTAM.

The Examiner appears to be asking the Applicant to enable a “gold standard” diagnostic test (ie the most definitive method available to establish the presence of a disease) (see Bachmann L.M. et al. at p.953 enclosed). A gold standard diagnostic test is often expensive and/or not possible without invasive surgery, and in some cases is only available post mortem. This does not diminish the utility of the methods claimed. Claims 49, and 58-65 merely require that the determinations resulting from the comparisons within the claim result in an indication of coronary artery disease. The use of a biomarker as an indicator of disease, is typically just one aspect of a multi-factorial process used for determining the likelihood that the person may have the disease of interest and so as to guide medical decisions regarding additional testing and treatment. For example, as noted in Stedman’s 27th Edition Medical Dictionary, “indication” is not equated with “diagnosis”. The term “*indication*” is understood to mean “**the basis for initiation** of a treatment for a disease or **of a diagnostic test**” (p. 892). Even a “*diagnostic test*” is not considered to result in an absolute certainty of a diagnosis – but rather is noted as “**relating to or aiding in diagnosis**”. As noted in Harrison’s Principles of Internal Medicine, Introduction to Clinical Medicine “the purpose of performing a test on a patient is to reduce uncertainty about the patient’s diagnosis or prognosis and to aid the clinician in making management decisions” (Ch I, pg. 11). This same text further notes that while “a perfect test would have a sensitivity of 100% and a specificity of 100% and would completely separate patients with disease from those without it...there are no perfect tests, after every test is completed the true disease state of the patient remains uncertain” (Ch I, pg. 11).

Furthermore, claims 49, and 58-65 require that the level of expression of the test subject be compared with the level of expression of control individuals having coronary artery disease and the comparison result in a statistically significant similarity for the determination to be indicative of coronary artery disease. Therefore, in order for the test to incorrectly indicate the presence of coronary artery disease in a test individual, there must be another disease state which results in a statistically significant similarity in the level of expression as compared to expression levels in coronary artery disease.

While Loughran JR, et al. notes a differential expression of either 3.9 fold or 11.8 fold for CRTAM of LGL patients compared with healthy individuals, Loughran **only looks at differential expression fractionated blood samples**, either in mononuclear cells (PBMCs) (see for example p.8 para. [0063], [0066]; p. 9 para. [0075] and [0076] and Table 2) or further fractionated leukocytes, namely, CD8+ T cells (see.p.8 para.[0066] and p.9 para [0077]. This is in contrast to the experiments performed by the Applicant which utilize RNA from blood samples which include RNA of all leukocytes, without any fractionating (paragraph [0240] of the Published Application). As noted in post-filing reference Du et al., each of the blood cell types can provides its own unique contribution towards a measured level of expression as between disease and control subjects (“several blood RNA isolation methods have been used to date...however, the RNA isolated using these methods comes from various blood cell subsets that originate from different developmental lineages, perform separate and distinct biological functions, and, most likely, have very different genomic expression signatures” (see p.701, 1st column of Du et al.)), therefore it is not known what the level of difference in expression would be in RNA from samples as claimed when comparing patients with LGL to control individuals and whether there would be a statistically significant similar level of expression as between patients having LGL and patients having coronary artery disease.

Similarly, the Examiner suggests that differential expression of CRTAM RNA could be an indicator of an immune response based on Zlontnik’s teachings that CRTAM protein is expressed on activated T-cells. As noted above, an increase in activated T-cells is only one of the cell types expressing RNA in whole blood. Furthermore, the teachings of Zlotnik are limited to demonstrating an increased cell surface expression of a protein on the surface of activated T-cells. The level of surface expression of a protein does not necessarily even correlate with increased protein translation levels since increased surface expression of the protein may merely be due to increased transport of pre-existing protein to the surface. Examples of such regulation abound in the art.

Therefore, the possibility that a person with, for example, with large granular lymphocyte leukemia might be falsely mischaracterized as having coronary artery disease, is highly unlikely. However, even if there was a statistically significant

similarity as between individuals having large granular lymphocyte leukemia and coronary artery disease (which the Applicant denies), this would not detract from the utility of the biomarkers as an indication of coronary artery disease. Rather such a hypothetical result would merely reduce the specificity of the biomarker, without affecting the sensitivity. Furthermore, the diseases of leukemia, and coronary artery disease have such different etiologies, and symptoms, it is highly unlikely that a person having large granular lymphocyte leukemia would be submitted for testing in accordance with the methods as claimed.

The office action also suggests that the Applicant has not taught that the elected gene alone “is sufficient to indicate the presence of coronary artery disease” (see p. 6 of the Office Action). The Applicant notes that by virtue of demonstrating a differential expression as between individuals having coronary artery disease and individuals not having coronary artery disease, they have demonstrated that the single gene is indicative of coronary artery disease. Furthermore, the Applicant has demonstrated, both within the specification, and in the Declaration that the differential expression of the elected gene is statistically significant as between individuals having coronary artery disease and individuals not having coronary artery disease – itself demonstrating that the elected gene is indicative of coronary artery disease. Finally, the Applicant notes that it is not aware of any teaching or suggestion of looking in blood for biomarkers indicative of coronary artery disease prior to applicant’s filing, and it is only as a result of the USPTO’s policy regarding restriction requirements that the Applicant has been forced to narrow the claims to a specific gene or set of genes. In light of the amendments and above remarks, the Applicant contends that the claims are fully enabled, and respectfully requests reconsideration and withdrawal of the instant rejections.

Conclusion

Applicant submits that all claims are allowable as written and respectfully request early favorable action by the Examiner. No new matter is added. If the Examiner believes that a telephone conversation with Applicant's attorney/agent would expedite prosecution of this application, the Examiner is cordially invited to call the undersigned attorney/agent of record.

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Respectfully submitted,

Amy DeCloux 54849 *Amy DeCloux*

Name: Kathleen Williams

Registration No.: 34, 380

Customer No.: 29933

Edward Angell Palmer & Dodge LLP

P.O. Box 55874

Boston, MA 02205

Tel: 617-239-0100

Encl.

- (a) Excerpts from Stedman, Thomas Lathrop, 1853-1938, Stedman's Medical Dictionary 27th
- (b) Excerpts from Harrison's Principles of Internal Medicine, ch I Introduction to Clinical Medicine, p. 11.
- (c) Immunobiology. Garland Publishing. 2001. Fifth Edition. Janeway, Travers, Walport, and Shlomchik, eds. Fig. A.23,
- (d) Bachmann L.M., Juni, P., Reichenbach, S., Ziswiler, H-R, Kessels, A.G., and Vogelin, E. "Consequences of different diagnostic 'gold standards' in test accuracy research: Carpal Tunnel Syndrome as an example." *International Journal of Epidemiology* 2005 34 953-955.

Abstract of: Casey et al., 1988. simplified plastic embedding and immunohistologic technique for immunophenotypic analysis of human hematopoietic and lymphoid tissues. Am J Pathol. 131:183-9

Routine fixation and paraffin embedding destroys many hematopoietic and lymphoid differentiation antigens detected by flow cytometry or frozen section immunohistochemistry. On the other hand, morphologic evaluation is difficult in flow cytometric or frozen section studies. A simplified three-step plastic embedding system using acetone-fixed tissues embedded in glycol-methacrylate (GMA) resin has been found to provide both excellent morphologic and antigenic preservation. With our system, a wide variety of antigens are detected in plastic sections without trypsinization or prolonged embedding procedures; **pan-B** (CD19, CD22), **pan-T** (CD7, CD5, CD3, CD2), T-subset (CD4, CD8, CD1, CD25) markers as well as surface immunoglobulin and markers for myeloid and mononuclear-phagocyte cells are preserved. In summary, modifications of plastic embedding techniques used in this study simplify the procedure, apparently achieve excellent antigenic preservation, and facilitate evaluation of morphologic details in relation to immunocytochemical markers.

Abstract of: Vilain A., Bernardino J., Gerbault-Seureau M., Vogt N., Niveleau A., Lefrancois D., Malfoy B., and Dutrillaux B. (2000) DNA methylation and chromosome instability in lymphoblastoid cell lines. Cytogen. Cell Genet. (2000) 90 (1-2) 93-101

In order to gain more insight into the relationships between DNA methylation and genome stability, chromosomal and molecular evolutions of four Epstein-Barr virus-transformed human lymphoblastoid cell lines were followed in culture for more than 2 yr. The four cell lines underwent early, strong overall demethylation of the genome. The classical satellite-rich, heterochromatic, juxtacentromeric regions of chromosomes 1, 9, and 16 and the distal part of the long arm of the Y chromosome displayed specific behavior with time in culture. In two cell lines, they underwent a strong demethylation, involving successively chromosomes Y, 9, 16, and 1, whereas in the two other cell lines, they remained heavily methylated. For classical satellite 2-rich heterochromatic regions of chromosomes 1 and 16, a direct relationship could be established between their demethylation, their undercondensation at metaphase, and their involvement in non-clonal rearrangements. Unstable sites distributed along the whole chromosomes were found only when the heterochromatic regions of chromosomes 1 and 16 were unstable. The classical satellite 3-rich heterochromatic region of chromosomes 9 and Y, despite their strong demethylation, remained condensed and stable. Genome demethylation and chromosome instability could not be related to variations in mRNA amounts of the DNA methyltransferases DNMT1, DNMT3A, and DNMT3B and DNA demethylase. These data suggest that the influence of DNA demethylation on chromosome stability is modulated by a sequence-specific chromatin structure. Copyright 2000 S. Karger AG, Basel.

Abstract of: Chu TM, Prostate Cancer-Associated Markers (1990) Immunol. Ser. 53:339-56.

Immunodiagnosis of prostate cancer is at a more advanced stage than that of most other tumors. Two well-known markers, prostatic acid phosphatase and prostate-specific antigen, have been used in the clinical management of patients. Prostate-specific antigen is a more sensitive and reliable marker than prostatic acid phosphatase. Serum prostate-specific antigen is effective in monitoring disease status, predicting recurrence, and detecting residual disease. Prostate-specific antigen is a tool for the histological differential diagnosis of metastatic carcinomas, especially in the identification of metastatic prostate tumor cells in distant organs and in the differentiation of primary prostate carcinoma from poorly differentiated transitional cell carcinoma of the bladder. Few data on biological function are available. Prostatic acid phosphatase functions as a phosphotyrosyl-protein phosphatase and prostate-specific antigen as a protease. Physiological function in the prostate remains to be elucidated. Several of the prostate-specific and prostate-tumor-associated antigens, as well as a putative prostate tumor-specific antigen, as recognized by monoclonal antibodies are available. Clinical evaluation of these potential markers is not yet available.

Abstract of Diamandis EP Prostate-specific antigen: a cancer fighter and a valuable messenger? Clin Chem. 2000 Jul; 46(7) 896:900

BACKGROUND: Prostate-specific antigen (PSA) is a valuable prostatic cancer biomarker that is now widely used for population screening, diagnosis, and monitoring of patients with prostate cancer. Despite the voluminous literature on this biomarker, relatively few reports have addressed the issue of its physiological function and its connection to the pathogenesis and progression of prostate and other cancers. **APPROACH:** I here review literature dealing with PSA physiology and pathobiology and discuss reports that either suggest that PSA is a beneficial molecule with tumor suppressor activity or that PSA has deleterious effects in prostate, breast, and possibly other cancers. **CONTENT:** The present scientific literature on PSA physiology and pathobiology is confusing. A group of reports have suggested that PSA may act as a tumor suppressor, a negative regulator of cell growth, and an apoptotic molecule, whereas others suggest that PSA may, through its chymotrypsin-like activity, promote tumor progression and metastasis. **SUMMARY:** The physiological function of PSA is still not well understood. Because PSA is just one member of the human kallikrein gene family, it is possible that its biological functions are related to the activity of other related kallikreins. Only when the physiological functions of PSA and other kallikreins are elucidated will we be able to explain the currently apparently conflicting experimental data.